COMPARISON OF BAIT MARKERS FOR BLACK RATS

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Abstract: Rodenticides are a necessary part of successful large-scale rodent control programs, but poor bait acceptance by rats compromises the effectiveness of many baiting programs. Non-toxic bait markers are one approach for assessing consumption and identifying the cause of failure. In single-feeding laboratory bioassays, we evaluated metallic flakes, Solvent Blue 36 oil-soluble dye, and tetracycline hydrochloride (THC) as bait markers for monitoring consumption of oat groats by captive black rats (*Rattus rattus*). We detected metallic flakes (0.3% [g/g] dietary concentration) in the gastrointestinal (GI) tracts of all 12 rats examined immediately following a single 24-hour feeding trial but in only 1 of 12 rats examined 48 hours later. We easily detected Solvent Blue 36 oil-soluble dye (0.1% to 0.5% [g/g]) in subcutaneous, abdominal, and genital fat of 7 of 8 rats 2 days after feeding, but found it in only 6 of 12 rats examined 5 days after feeding. Dye applied at 1.0% and 1.5% (g/g) persisted in all 8 rats inspected 5 days after feeding, but reduced consumption ($P < 0.05$) at these levels suggests that concentrations ≥1% are unpalatable to rats. Dietary concentrations of THC from 0.5% to 1.0% (g/g) produced a golden-yellow fluorescence under ultraviolet (UV) illumination on the incisors and growing points of the mandibles of all rats examined 3 and 14 days after feeding. Because THC had no apparent effect on consumption ($P = 0.28$), it offers a reliable means of detecting consumption by black rats and thus, of determining whether poor results with rodenticides are due to poor bait acceptance.

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Bait markers are valuable for studying the ecology and feeding behavior of free-ranging animals (Savarie et al. 1992). They have been used to monitor consumption (New 1958, Nass and Hood 1969, Saunders et al. 1993), study movements (Gast 1963, Frantz 1972), determine population structure (Klevezal and Mina 1984), and assess nontarget hazards of pesticide baiting programs (Lindsey et al. 1979). Bait markers include dyes (New 1958, 1959; Gast 1963; Nass and Hood 1969; Lindsey et al. 1979; Yescott 1978), fluorescent pigments (Frantz 1972, Evans and Griffith 1973, Johns and Pan 1982), antibiotics (Linhart and Kennelly 1967, Crier 1970, Lefebvre et al. 1987), and inert particles (Milan and Jackson 1987, Fall and Johns 1987).

Black rats cause substantial damage in Hawaiian macadamia (*Macadamia integrifolia*) orchards (Tobin et al. 1993, 1994). Many macadamia growers in Hawaii use rodenticides to reduce pest populations, although current methods of rodenticide application are only marginally effective in controlling damage (Tobin unpubl. data). Regulations allow baits to be placed in burrows, put in bait stations in trees, or broadcast across the orchard floor. Managers typically choose the latter option because it is the fastest and least labor intensive. However, in some orchards rats spend most of their active hours in the canopy, and thus may not consume bait placed on the ground (Tobin et al. unpubl. data).

To determine the percentage of black rats that consume bait placed in burrows, in trees, and on the ground, managers need a reliable marker to distinguish rats that ingested bait placed in these locations. Thus, we evaluated 3 bait markers for detecting consumption by black rats: inert metallic flakes, an oil-soluble dye, and the antibiotic THC. Our objectives were to determine the percentage of rats marked with various colors and the concentrations of these 3 bait markers at various intervals after feeding.

M. W. Fall, P. J. Savarie, and B. E. Johns commented on an earlier draft of this manuscript. We captured, maintained, and tested rats according to U.S. Department of Agriculture, Denver Wildlife Research Center approved standard operating procedures and animal welfare standards.

METHODS

Test Animals

We captured black rats in Haguruma® Japanese cage traps baited with chunks of coconut in forested areas near Hilo, Hawaii (reference to commercial products is for identification only and does not imply endorsement by the authors or the U.S. Department of Agriculture). We checked traps daily, transported captures to the Denver Wildlife Research Center's field station in Hilo, dusted rats with carbaryl powder, and individually housed them in 18- x 18- x 36-cm stainless steel, wire mesh cages in rooms with ambient temperature of 25 C and a 12-hour light/12-hour dark cycle. We maintained rats on an ad libitum diet of rodent laboratory chow (Purina Mills, Inc., St. Louis, Mo.) and water and held them in quarantine for a minimum of 21 days before testing. At the end of quarantine, we weighed animals that had no visible injuries or signs of illness, and transferred adults weighing >90 g to another room for testing.

General Procedures

We prepared all baits using slightly crimped oat groats (La Crosse Milling Company, Cochrane, Wis.) that had been sifted through a 20-mm mesh screen to remove fine debris. We heated Alcolec-S® (American Lecithin Company, Inc., Atlanta, Ga.) in a water bath to decrease its viscosity, drew it into a syringe, and mixed it with oats as an adhesive.

Before testing, we offered rats untreated oats ad libitum in addition to rodent laboratory chow for 3–11 days. We hung cages in alternate spaces on racks to minimize contamination between cages. The morning of the feeding trial, we replaced the untreated oats and laboratory chow with the appropriate test bait, and placed trays under the cages to collect spillage. Based on laboratory feeding trials, we restricted the amount of bait offered to each rat to simulate levels of toxic bait likely to be consumed in the field. Twenty-four hours after offering the test bait, we removed and weighed uneaten bait and any spillage, and moved the animals to another rack with clean cages and fresh laboratory chow. We calculated consumption as the amount of food offered minus the amount remaining and any spillage. We performed analyses of variance and Duncan's multiple range tests with an experiment-wise error rate of 0.05 to compare consumption among groups. At selected intervals after completion of the feeding trials, we administered a lethal dose of CO_2 to randomly selected rats and examined them for markings. We disposed of carcasses at the Hawaii County animal disposal site.

Metallic Flakes Trial

Baits consisted of 0.3% (g/g) Glowble® metallic flakes (Metalflake, Inc., Haverhill, Mass.), 2.0% Alcolec-S, and 97.7% oats. We used 3 colors of flakes: Glowble Bright Red, Glowble Royal Blue, and Glowble Nile Green. We mixed the Alcolec-S with the oat groats for 5 minutes, added and mixed in the metallic flakes for an additional 5 minutes, and let the bait dry overnight.

We randomly assigned 6 rats of each sex to each of the color groups. We offered 2.0 g of bait to each of 3 rats per sex and color, and 5 g to the remaining rats. From each color and quantity of bait, we randomly selected 1 male and 1 female for examination 0, 24, and 48 hours after completion of the feeding trial. We removed the GI tract of each rat and expressed its contents into petri dish covers by pulling the intestines between 2 fingers and by slicing the stomach and caecum open to rinse out the contents. With water, we removed any metallic flakes from the dissecting equipment and diluted the contents in the petri dish cover. We broke apart and spread out the stomach contents to form a uniform, thin layer. We then inserted the bottom of the petri dish and pressed down to facilitate examination of the diluted GI contents.

Oil-soluble Dye Trial

We mixed oats with 1.25% (g/g) Alcolec-S and 1 of 5 concentrations of Solvent Blue 36 oil-soluble dye (Pylam Products Company, Garden City, N.Y.): 0.1, 0.3, 0.5, 1.0, and 1.5% (g/g). For each bait, we coated the oats with about 2/3 of the Alcolec-S, added the appropriate amount of dye, and coated the bait with the remaining Alcolec-S. We mixed the bait for 5 minutes after each addition.

We randomly assigned 4 rats of each sex to each of the 5 concentration groups and offered each animal 5 g of bait for 24 hours. We inspected all rats for signs of the dye around the mouth, anus, ears, and paws. Two and 5 days after the trial, we sacrificed 2 randomly selected rats per sex per group and examined them for markings. We made an incision along the ven-

Table 1. Consumption and number of black rats marked at 2 and 5 days after a single 24-hour exposure to 5 g oats containing Solvent Blue 36 dye. Two rats of each sex were randomly assigned to each concentration/sampling day combination.

Dye level (%, g/g)	Consumption				Days after feeding	Marked rats	
	Bait (g)		Dye (mg/kg)				
	$\bar{x}$[a]	SE	$\bar{x}$[a]	SE		External[b]	Internal[c]
0.1	4.9	0.03	31.6	1.42	2	1	3
0.1	4.8	0.02	30.9	2.36	5	0	2
Pooled	4.8[A]		31.3[A]				
0.3	3.1	0.66	54.4	11.19	2	2	4
0.3	4.4	0.30	85.2	10.15	5	0	2
Pooled	3.7[A]		69.8[AB]				
0.5	4.2	0.43	142.5	19.54	2	3	4
0.5	3.2	0.84	93.9	22.29	5	0	2
Pooled	3.7[A]		118.2[BC]				
1.0	3.0	0.45	187.0	20.93	2	3	4
1.0	2.1	0.20	142.8	16.70	5	1	4
Pooled	2.5[B]		164.9[C]				
1.5	1.8	1.00	165.4	89.47	2	1	3
1.5	1.7	0.28	181.7	40.02	5	1	4
Pooled	1.8[B]		173.5[C]				

[a] Pooled means that share a common letter in each column do not differ ($P > 0.05$) based on Duncan's multiple range test.
[b] Dye most often was visible around the vaginal, urinary, or anal openings, under the fur on the ventral surface, or at the base of the ears.
[c] Dye most often was present in subcutaneous, abdominal, or genital fat.

tral midline from the pelvis to the neck and inspected the fat and internal organs for discoloration due to absorption of the dye.

THC Trial

We prepared baits with 0.5, 0.75, and 1.0% (g/g) THC (Schweizerhall, Inc., South Plainfield, N.J.) and 2.0% (g/g) Alcolec-S. We coated the oats with approximately 1/2 of the Alcolec-S before adding the appropriate amount of THC. We mixed baits for 5 minutes after each addition. We inspected the oats under a long-wave UV light to confirm that the THC was distributed uniformly, and then added the remaining Alcolec-S and mixed for 5 additional minutes. We placed baits in beakers, enclosed in black plastic bags to minimize exposure to light, and refrigerated the baits at 10 C until used.

We randomly assigned 6 rats of each sex to each concentration group and offered them 5 g of bait for 24 hours. We sacrificed 2 randomly selected rats of each sex and concentration 3, 7, and 14 days after the feeding trial. In addition, for reference on day 3 we sacrificed 2 males and 2 females not exposed to THC. We examined the exposed portion of the incisors under a long-wave (3150–4000 Å) UV light for fluorescence, and then extracted and boiled the lower mandibles in water to easily remove flesh and incisors. We cleaned and examined under a UV light the mandibles and lower incisors to detect fluorescence at the growing points.

RESULTS

Metallic Flakes

Except for minor spills, every rat consumed all the bait that it was offered. Immediately after the feeding trial, we detected metallic flakes throughout the GI tracts of all rats inspected, regardless of the amount of bait eaten or the color of flake. The flakes in the stomach, intestines, and feces appeared unaltered by passage through the GI tract. At 24 hours, only 1 rat offered 2 g of bait, but all rats that were offered 5 g of bait, still had flakes in their GI tracts. In all instances, flakes were scarce enough so that the number of individual particles could be counted. By 48 hours, the GI tract of the lone-marked rat had a single metallic flake.

Oil-soluble Dye

Both consumption of bait ($F = 10.15$; 4, 35 df; $P = 0.0001$) and ingestion of dye per kg body mass ($F = 7.04$; 4, 35 df; $P = 0.0003$) varied among concentration groups (Table 1). Rats in the 2 highest concentration groups consumed less bait ($P < 0.05$) but ingested more dye per kg body mass ($P < 0.05$) than did rats in any other group. Rats in the 0.5% group ingested more dye per kg body mass than did rats in the 0.1% group ($P < 0.05$). Ingestion of dye by rats in the 0.3% group did not differ from that of rats in either the 0.1% or 0.5% groups ($P > 0.05$).

Immediately after the 24-hour feeding trial,

Table 2. Consumption and number of black rats marked at 3, 7, and 14 days after a single 24-hour exposure to 5 g oats containing tetracycline hydrochloride (THC). Two rats of each sex were randomly assigned to each concentration/sampling day combination.

THC level (%, g/g)	Consumption				Days after feeding	Marked rats	
	Bait (g)		THC (mg/kg)				
	$\bar{x}$[a]	SE	$\bar{x}$[b]	SE		External[c]	Internal[d]
0.50	4.4	0.1	149.6	16.1	3	3	4
0.50	3.9	0.6	111.2	14.7	7	2	4
0.50	4.4	0.3	146.0	23.6	14	4	4
Pooled	4.2		135.6[A]				
0.75	4.3	0.2	197.9	19.6	3	3	4
0.75	4.3	0.4	194.6	29.1	7	3	4
0.75	4.8	0.1	241.1	16.1	14	4	4
Pooled	4.5		211.2[B]				
1.00	3.6	0.4	235.8	35.4	3	4	4
1.00	4.4	0.2	276.4	41.8	7	3	4
1.00	4.1	0.2	248.7	29.0	14	4	4
Pooled	4.0		253.6[B]				

[a] Consumption did not vary among concentrations ($F = 1.33$; 2, 33 df; $P = 0.28$).
[b] Pooled means that share a common letter in each column do not differ ($P > 0.05$) based on Duncan's multiple range test.
[c] On the exposed portion of incisors above the gingival margin.
[d] On the condyle and coronoid process of mandibles or on the unexposed portion of incisors below the gingival margin.

32 of 40 rats (5–8/group) had externally visible signs of the dye. Most common were bluish markings around the anal, urinary, and vaginal openings, and bluish internal stains visible through the ventral surface and at the base of the ears. One to 3 rats in each group still had externally visible markings 2 days later, but by day 5 only 1 rat in each of the 2 highest concentration groups still showed external evidence of ingesting dye (Table 1).

All rats with externally visible signs were marked internally. Two days after the trial, all except 2 rats had subcutaneous, abdominal, or genital fat deposits stained blue. One of the rats without markings was in the lowest concentration group, and the other, although in the highest concentration group, had the lowest bait consumption (0.29 g) of all the rats tested. By day 5, the incidence and intensity of dye in fat deposits declined, but we still detected pale blue fat deposits in half of the rats in the 3 lowest groups and all 4 rats in each of the 2 highest groups.

THC

Whereas total consumption of the THC-treated bait did not vary among concentrations ($F = 1.33$; 2, 33 df; $P = 0.28$), THC ingested per kg body mass did ($F = 15.93$; 2, 33 df; $P = 0.0001$). Rats offered the 0.75% and 1.0% baits ingested more THC than did rats offered the 0.5% THC bait ($P < 0.05$) (Table 2).

Three days after the feeding trial, the mandibular condyle and coronoid process of all mandibles inspected, as well as the base of all excised incisors, had the golden-yellow fluorescence characteristic of antibiotic markers. The fluorescence covered a progressively larger area on the exposed portion of incisors, and was externally visible in all rats by day 14.

DISCUSSION

Different colored metallic flakes are distinguishable in the same animal (Fall and Johns 1987), and therefore can be used to evaluate >1 treatment (e.g., multiple food sources or feeding locations) in the same study area simultaneously. Although we examined only the GI tracts, Fall and Johns (1987) conducted similar feeding trials and detected metallic flakes in the feces of rats, indicating that metallic flakes may provide a nonlethal means of detecting feeding by rats.

Metallic flakes persist in the alimentary tract for only a limited period, however. Milan (1985) reported that metallic flakes remained visible in the gut and feces of Philippine rice-field rats (*R. rattus mindanensis*) for 6 days after a 7-day exposure to rice bait marked with metallic flakes. Fall and Johns (1987) exposed laboratory rats (*R. norvegicus*) to cornmeal treated with Glowble metallic flakes and found 100% of rats marked at the completion of a 24-hour feeding trial, 50% marked 2 days after feeding, and no evidence of markers 1 week after feeding. These latter findings are similar to ours and indicate that metallic flakes consumed during ≤24 hours

pass through the GI tract too rapidly to serve as a reliable marker of consumption by black rats ≥2 days after ingestion.

The lack of externally visible evidence in most rats with internal markings of Solvent Blue 36 oil-soluble dye indicates the necessity of dissecting rats to examine their fat deposits. We did not determine whether the dye colored feces sufficiently to indicate bait consumption.

The decline between the second and fifth day in the proportion of rats marked with the 3 lowest concentrations of Solvent Blue 36 oil-soluble dye indicates that this dye is excreted rapidly. Thus, baits formulated with this dye at concentrations ≤0.5% probably would not be reliable indicators of bait consumption by rats >2 days after feeding. Yescott (1978) also observed rapid elimination of an oil-soluble dye from the fat of rats.

In the 2 highest concentration groups, dye remained in the fat for at least 5 days, but reduced consumption suggests that concentrations ≥1% are unpalatable to rats and would compromise the results of field studies. Oil-soluble dyes vary in their persistence and visibility in biological systems (Larthe 1958, New 1959), and other dyes may be more suitable for studying consumption by rats.

THC was the most reliable indicator of feeding by black rats and had no apparent effect on consumption. The persistence of this marker permits flexibility in time of collection of animals for examination. The appearance of fluorescence above the gingival margin as the incisors grew indicates that researchers capturing animals ≥14 days after treatment need not dissect animals to observe markings, but can examine the external portion of the incisors on dead or anesthetized rats. Further study is needed to determine when the fluorescence first appears above the gingival margin and how long it persists.

MANAGEMENT IMPLICATIONS

Rats (*Rattus* spp.) cause major health (Gratz 1988), economic (Dubock 1984) and ecological (Moors et al. 1992) problems throughout the world, and in many cases rodenticides are the only practical control method available (Jackson 1987, Myllymäki 1987). Many rodenticide baiting programs fail, however, due to neophobia, bait shyness, improper bait placement, and resistance (Barnett 1988, Prakash 1988). Identifying causes of bait failure is difficult, but crucial if the effectiveness of baiting programs is to be improved. THC is a reliable, non-repellent marker that researchers and managers can use to determine the degree of bait acceptance. THC will enable Hawaiian macadamia growers and others to interpret inconsistent results with rodenticides, and lead to improved baiting techniques.

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